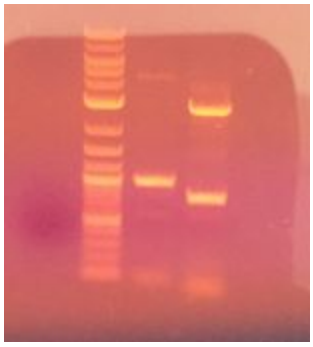
Friday, July 1, 2016 (Christine Tang, Margaret Lie, Peter Suzuki)

Objectives: Gel Verification of Hypoxia-670-Violacein Vector to see if PCR reaction is wrong, and to PCR of hypoxia 670 and iRFP 713 to golden gate

1. Gel verification of the backbone using a 0.8% agarose gel.



2. PCR of hypoxia-iRFP 670 and iRFP 713

*PCR setups for hypoxia-iRFP Golden Gates

Material	Volume
ThermoPol Buffer	5 uL
dNTPs	1 uL
Fwd Primer (iGEM 45 for hyp670, iGEM 47 for 713)	2.5 uL
Rev Primer (iGEM 46 for 670, iGEM 48 for 713)	2.5 uL
VentR DNA Polymerase	1uL
5 ng Template: hypoxia 670 #3 1:100 dilution .427 ng/uL or iRFP 713 #8 1:100 dilution .290 ng/uL	11.7 uL 670 17.2 uL 713
QS 50 uL dH2O	26.3 uL 670 21.8 uL 713

Vent Thermocycling conditions (on Benchling), 59.5C (2201 bp) for 670, 58.9C (982 bp) for 713.

3. PCR violacein

Material	Volume
ThermoPol Buffer	5 uL
dNTPs	1 uL
Fwd Primer (iGEM 19 for VioC, iGEM 12 for Vio ABDE ribozyme)	2.5 uL
Rev Primer (iGEM 20 for VioC, iGEM 13 for VioABDE ribozyme)	2.5 uL
VentR DNA Polymerase	1uL
5 ng Template	
QS 50 uL dH2O	

Vent Thermocycling conditions (on Benchling), 56.6, 1343 bp for VioC, 52.9, 8243 bp Vio ABDE ribozyme.

All samples stored at 4 degrees C after PCR for gel verification on July 5th.

Tuesday, July 5th, 2016 (Peter Suzuki, Christine Tang)

Objective: PCR iRFP 713 to put into SoxR/S **(Peter Suzuki)**

*Make IRFP 713 BioBrick Compatible:
Procedure:
Set up PCR reactions of pBAD/HisB-iRFP713 and Sox R/S.

Tube 1 (iRFP 713 1:100):

Material	Volume	Concentration
dH20	32.5 QS 50µL	-
High GC 5x Phusion HF Buffer	10 µL	-
dNTPs (50x)	1 µL	10 mM
iGEM 43	2.5 µL	10 µM
iGEM 44	2.5 µL	10 µM
Template	Variable (~1uL)	2-10 ng

Phusion DNA Polymerase (100x)	1 µL	100x stock

Thermocycling:

Temp. (°C)	Time	Cycle
98	0:30	
98	0:10	
61	0:30	25-35X
72	1:30	
72	10:00	
4	∞	

*Round down Tm for lowest annealing temperature primer

Tube 2 (Sox R/S 1:100):

Material	Volume	Concentration
dH20	32.5 QS 50µL	-
High GC 5x Phusion HF Buffer	10 µL	-
dNTPs (50x)	1 µL	10 mM
iGEM 42	2.5 µL	10 µM
iGEM 45	2.5 µL	10 µM
Template	Variable (~1uL)	2-10 ng
Phusion DNA Polymerase (100x)	1 µL	100x stock

Thermocycling:

Temp. (°C)	Time	Cycle
98	0:30	
98	0:10	
59.7	0:30	25-35X
72	1:00	
72	10:00	
4	∞	

*Round down Tm for lowest annealing temperature primer

2. Run 5 microliters of PCR product on gel made of 0.8% agarose. 70-80 V
3. Add 1 microliter Dpn 1 to rest of PCR product. Incubate 1 h at 37C.
4. Zymo Clean and Concentrate. Then check DNA concentration with nanodrop.
5. Golden Gate DNA Synthesis

Reaction Setup

X µl	Largest DNA piece (~100 ng)
X µl	each additional DNA assembly piece (equimolar)
1.5 µl	10x NEB T4 Buffer
1.5 µl	10x BSA*
0.5 µl	BsaI
0.5 µl	NEB T4 Ligase, 400.000 cohesive end units/ µl
QS 15 µl	dH2O

*Without the addition of BSA *Bsa*I is only 10% active at 37°C

Thermo Cycling

Temp. (°C)	Time	Cycle
37	3:00	
16	4:00	25x
50 (final digestion)	5:00	
80 (heat inactivation)	5:00	

6. Zymo DNA Clean and Concentrate
7. Transform 6 microliters into 1 tube (approx. 40 microliters) of XL1 E. coli by electroporation.
8. Incubate 1 h in 1 mL SOC at 37C, 250 rpm.
9. Plate 100 microliter, 1:100 dilution, and 1:1000 dilution on LB/Amp(100).
10. Incubate at 37C for 16 h.

Objective: Gel Verification of PCR Products, Hypoxia-670 and iRFP 713 **(Christine Tang)**

1. Ran 0.8% gel for both at their normal and 1:100 concentrations
2. Ran a DpnI digest for all 4, Zymocleaned.

Final concentrations: hypoxia-670: 108.8 ng/uL. iRFP 713: 68.2 ng/uL. Hypoxia 670 1:100: 65.4 ng/uL. 713 1:100: 19.8 ng/uL

Wednesday, July 6th 2016 (Margaret Lie)

Objective: To run a PCR to put SoxR/S and iRFP713 together, and hypoxia and iRFP 713 together. Then to run a gel verification of 713, SoxR/S, hypoxia713

1. Set up PCR reaction for Hypoxia + iRFP713.

Reaction Setup

37.5 µl	dH2O
5 µl	Thermopol Buffer
1 µl	dNTPs
2.5 µl	iGEM 45
2.5 µl	iGEM 46
1 µl	Hypoxia iRFP670 #3
1 µl	Vent R DNA polymerase

Thermocycling:

Temp. (°C)	Time	Cycle
95	5:00	
95	0:30	
61.4	0:30	25-35X
72	2:14*	
72	10:00	
4	∞	

*2218 bp

2. Set up PCR reaction for SoxR/S + iRFP713.

Reaction Setup

37.5 µl	dH2O
5 µl	Thermopol Buffer
1 µl	dNTPs
2.5 µl	iGEM 47
2.5 µl	iGEM 48
1 µl	iRFP713 #8

1 µl	Vent R DNA polymerase

Thermocycling:

Temp. (°C)	Time	Cycle
95	5:00	
95	0:30	
58.9	0:30	25-35X
72	0:59*	
72	10:00	
4	∞	

*982 bp

3. Ran 2 hypoxia samples, 4 713 samples, 3 Sox R/S samples on an 0.8% agarose gel.
4. Gel extracted 713 and Sox R/S using Zymo kit.
    a. Concentration of iRFP 713: 39.5 ng/uL
    b. Concentration of SoxR/S: 76.9 ng/uL

Objective: To run PCR of Violacein C parts 1 and 2 and the Violacein backbone for eventual ligation into single plasmid.

1. PCR setup after resuspending gblocks:

Reaction Setup (VioC part 1)

37.5 µl	dH2O
5 µl	Thermopol Buffer
1 µl	dNTPs
2.5 µl	iGEM 19b
2.5 µl	iGEM 49
0.5 µl	VioC Part 1
1 µl	Vent R DNA polymerase

Thermocycling:

Temp. (°C)	Time	Cycle

95	5:00	
95	0:30	
60.8	0:30	25-35X
72	0:45*	
72	10:00	
4	∞	

*750 bp

Reaction Setup (VioC part 2)

37.5 µl	dH2O
5 µl	Thermopol Buffer
1 µl	dNTPs
2.5 µl	iGEM 50
2.5 µl	iGEM 20
0.5 µl	VioC part 2
1 µl	Vent R DNA polymerase

Thermocycling:

Temp. (°C)	Time	Cycle
95	5:00	
95	0:30	
59.3	0:30	35X
72	0:38*	
72	10:00	
4	∞	

*622 bp

Reaction Setup (VioC backbone)

32.5 µl	dH2O
5 µl	Thermopol Buffer
1 µl	dNTPs
2.5 µl	iGEM 13
2.5 µl	iGEM 12

1 µl	VioC backbone #5
1 µl	Vent R DNA polymerase

Thermocycling:

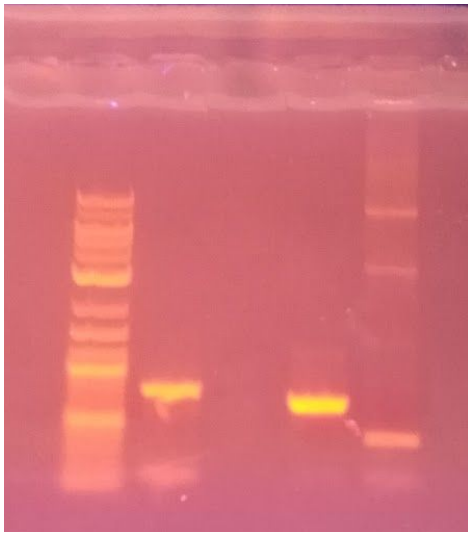
Temp. (°C)	Time	Cycle
95	5:00	
95	0:30	
60.4	0:30	25-35X
72	4:20*	
72	10:00	
4	∞	

*8393 bp

Thursday, July 7, 2016 (Peter Suzuki, Margaret Lie, Christine Tang)

Objectives: To do gel verification of VioC1, VioC2 and a gel extraction of VioBB (backbone). **(Peter Suzuki, Christine Tang)**

1. Ran a gel at 0.8% agarose of VioC1, VioC2 and VioBB (5 µl).



2. Dpn1 digested VioC1 and VioC2 for 1 hour at 37 degrees Celsius
3. Zymocleaned VioC1 and VioC2
4. Ran gel of VioBB (backbone) because only ran 5 µl in the previous gel. Used 0.8% agarose and ran at 80 V.

5. Gel extracted and zymocleaned.
6. Nanodropped to find the concentrations of VioC1, VioC2 and VioBB after zymoclean.
    a. VioC1 - 172.5 ng/µl
    b. VioC2 - 206.7 ng/µl
    c. VioBB - 22.2 ng/µl

Objectives: To golden gate VioBB, VioC part 1 (VioC1) and VioC part 2 (VioC2) together. To golden gate SoxR/S and iRFP713 together.

1. Set up Golden Gate reaction for VioBB, VioC1 and VioC2.

Reaction Setup

100 ng	VioBB
9 ng	VioC1
8 ng	VioC2
1.5 µl	T4 Ligase Buffer
1.5 µl	10x BSA
1 µl	*BsaI*
1 µl	T4 Ligase
QS 15 µl	dH2O

Thermocycling:

Temp. (°C)	Time	Cycle
37	3:00	

16	4:00	25x
50 (final digestion)	5:00	
80 (heat inactivation)	5:00	

2. Set up Golden Gate reaction for SoxR/S and iRFP713.

Reaction Setup

100 ng	SoxR/S PCR
35.3 ng	iRFP713 PCR
1.5 µl	T4 Ligase Buffer
1.5 µl	10x BSA
1 µl	*BsaI*
1 µl	T4 Ligase
QS 15 µl	dH2O

Thermocycling:

Temp. (^{o}C)	Time	Cycle
37	3:00	
16	4:00	25x
50 (final digestion)	5:00	
80 (heat inactivation)	5:00	

Objective: To make and store electrocompetent MG1655 *E. coli*. This is a two day process. **(Margaret Lie)**

Day 1:

1. Autoclaved 500 mL Luna Broth, 1 L 10% glycerol and plastic bottles (at least 2)
    a. LB: 5 g tryptone, 5 g NaCl, 2.5 g yeast extract. pH to 7.1 (start with 50 µl 10 N NaOH). QS to 500 mL H2O stirring.
    b. 10% glycerol: 100 mL glycerol and 900 mL milliQ H2O.
2. Incubated 5 mL culture of MG1655 in LB at 37 degrees Celsius for 18 hours, 250 rpm.

Friday, July 8, 2016 (Margaret Lie, Peter Suzuki, Christine Tang)

Objective: To finish making MG1655 *E. coli* (day 2).

Day 2:

1. Transferred 5 mL culture to 500 mL LB.
2. Incubated 2 hours at 37 degrees Celsius, 250 rpm.
3. Measured OD600 = 0.8.
    a. Too high but continued!
    b. Ideal was 0.5-0.7
    c. Remember to blank with 1 mL LB before measuring OD600
4. Put 500 mL culture into 2 plastic centrifuge bottles and glycerol on ice for 20 minutes.
5. Poured 250 mL culture in each plastic bottle. Balanced. Put in canisters.
6. Centrifuged at 6000 *xg* for 5 minutes at 4 degrees Celsius.
7. Decanted in flask and tapped on paper towel to remove liquids.
8. Resuspended each bottle in 250 mL 10% glycerol.
9. Centrifuged at 4000 *xg* for 15 minutes at 4 degrees Celsius.
10. Decanted as in step 7.
11. Resuspended each bottle in 125 mL 10% glycerol. Repeated step 9. Repeated step 7.
12. Resuspended in 10 mL 10% glycerol. Transferred to 50 mL centrifuge tube. Centrifuged 6000 *xg* for 5 minutes at 4 degrees Celsius.
13. Obtained 2 L liquid nitrogen with Shirley Liu at Chem Store. Also labeled 50 1.5 mL centrifuge tubes with MG1655. Put box in -80 degrees Celsius and tubes on ice
14. Repeated step 7. Resuspended in 1 mL 10% glycerol. Aliquoted 40 µl cells and flash froze in liquid nitrogen.
15. Ladelled into box. Stored at -80 degrees Celsius (Row 2 column 2).

Monday, July 11th, 2016 (pg 22)

Objective: To start arabinose assay w/ Hemoxygenase (day 1).

a. Grew Cultures for iRFP 670 and iRFP 713.

Objective: Making hypoxia 670 Biobrick Compatible

Phusion PCR Master Mix:

a.

Material	Volume	Concentration
dH20	32.2 QS 50µL	-
5x Phusion Buffer	10 µL	-
dNTPs (50x)	1 µL	10 mM

iGEM 17b (20x)	2.5 µL	10 µM
iGEM 18b (20x)	2.5 µL	10 µM
#1 hypoxia 670 template	1.3 uL	
Phusion DNA Polymerase	0.5 µL	100x stock

b. Thermocycling:

Temp. (°C)	Time	Cycle
98	0:30	
98	0:10	
58.4	0:30	35X
72	0:30	
72	10:00	
4	∞	

Objective: Colony PCR of VioC and Sox R/S+713

Procedure:

1. Made Vent Master Mixes for each PCR
   a. <u>VioC</u>

Material	Volume	Concentration
dH20	33.5 QS 50µL	-
ThermoPol Buffer	5 µL	-
dNTPs (50x)	1 µL	10 mM
iGEM 19b (20x)	2.5 µL	10 µM
iGEM 20 (20x)	2.5 µL	10 µM
Vent DNA Polymerase	0.5 µL	100x stock

   b. <u>Sox R/S 713</u>

Material	Volume	Concentration
dH20	33.5 QS 50µL	-
ThermoPol Buffer	5 µL	-
dNTPs (50x)	1 µL	10 mM
VF2 (20x)	2.5 µL	10 µM
VR (20x)	2.5 µL	10 µM
Vent DNA Polymerase	0.5 µL	100x stock

   B. Referred to colony PCR procedure for rest of procedure.

2. Thermocycling:

Temp. (°C)	Time	Cycle
95	0:30	
95	0:30	
56.6 for		

vioC and
51.6 for
SoxR/S/713 0:30 35X
68 0:39 for VioC / 1:00 for SoxR/S/713
68 5:00
4 ∞
3. Incubated plates at 37 degrees Celsius.
4. Incubated cultures in the 37 degrees Celsius shaker. Made 4 3mL cultures each of VioC and soxR/S/713.

Summary: Will miniprep and send for sequencing tomorrow.

Tuesday, July 12th, 2016

Objective: Begin preparing arabinose assay with hemeoxygenase (Day 1)

Procedure:

1. Arabinose added to cells as follows
    a. 8 trials each of:
        i. 50 mM
        ii. 20 mM
        iii. 10 mM
        iv. 1 mM
        v. 100 uM
        vi. 10 uM
        vii. 1 uM
        viii. 100 nM
        ix. 10 nM
        x. 1 nM
        xi. 0.1 nM
        xii. No arabinose (control)
2. Cells transferred to 96-well plates at log phase

Objectives: Miniprep and Sequencing of VioC and SoxR/S 713 samples from July 11

Procedure:

1. Miniprep of VioC (samples 1,2, and 4) - no growth in sample 3
2. Miniprep of SoxR/S (samples 5,6,7, and 8)
3. Nanodrop and mailing for sequencing

Sample	Concentration/Amounts Needed	H2O needed
VioC1	500 ng/(266 ng/uL) = 1.9 uL	11.1 uL
VioC2	500 ng/(226.2 ng/uL) = 2.2 uL	10.8 uL
VioC4	500 ng/(180.4 ng/uL) = 2.8 uL	10.2 uL
SoxR/S 5	500 ng/(206.2 ng/uL) = 2.4 uL	10.6 uL
SoxR/S 6	500 ng/(179.1 ng/uL) = 2.8 uL	10.2 uL
SoxR/S 7	500 ng/(292.2 ng/uL) = 1.7 uL	11.3 uL
SoxR/S 8	500 ng/(170.7 ng/uL) = 2.9 uL	10.1 uL

Wednesday, July 13th, 2016

Objectives:

- Finish arabinose induction assay
- PCR biobrick compatible iRFP 713 → put into constitutive promoter
- Set up golden gate for hypoxia 670 to make biobrick compatible
- Order primers for Tet plasmid
- Analyze sequencing results

Arabinose Induction Assay

1. With 200 uL multichannel pipet, transferred cells to 96-well plate
2. Centrifuged with rotor 5.3, 3000xg for 5min @4 C
3. Discarded supernatant
4. Used TECAN Infinite M1000 Plate Reader
    a. Opened iRFP 670 program
    b. Used Costar 96 Round Transparent for plate definition
    c. Highlighted entire plate
    d. Used 600 nm absorbance, 50 flashes, excitation of 643 nm, manual gain
    e. For fluorescence intensity scan, used excitation wavelengths from 600-700nm with 2 nm step size
    f. For excitation scan, used emission wavelengths from 650-750 nm

Making hypoxia 670 BioBrick compatible

1. Nanodrop DNA - 102.3 ng/uL
2. Set up Golden Gate assembly

1 ul	Hypoxia 670 biobrick compatible
1.5 ul	10x NEB T4 Buffer
1.5 µl	10x BSA
1 µl	*BsaI*
1 µl	NEB T4 Ligase
QS 15 µl	dH2O

3. ZymoClean of DNA product
4. Transformed electrocompetent bacteria with DNA product
5. Plated 2 plates with transformation, one 100uL one with remaining volume

Adding Constitutive Promoter to BioBrick iRFP 713

Material	Volume	Concentration
dH20	36.8 QS 50µL	-
5x ThermoPol Buffer	10 µL	-
dNTPs (50x)	1 µL	10 mM
iGEM 60 (20x)	2.5 µL	10 µM
iGEM 61 (20x)	2.5 µL	10 µM
pBAD/His/713 template	1.7 uL	
VentR DNA Polymerase	0.5 µL	100x stock

Thermocycling:

Temp. (°C)	Time	Cycle
95	5:00	
95	0:30	
60.5	0:30	35X
72	1:00	
72	10:00	
4	∞	

Analysis of Sequencing

- VioC 1 and 2 are bad; need to be re-done
- Sox5 ok
- Sox6 good

- Sox7 good
- Sox8 ok

Thursday, July 14th, 2016

Objective: Designing VioC primers

1. Made iGEM 65 and iGEM 66 on Benchling

Objective: Gel Verification of Constituitive Promoter with 713

1. Ran a 0.8% agarose gel of the 2 samples from July 13 at 110 V for 20 minutes.

Results: There were no bands at all, so did not proceed with Zymoclean. Thermopol buffer or dNTP stocks may have been contaminated, so trashed them.

Friday, July 15, 2016

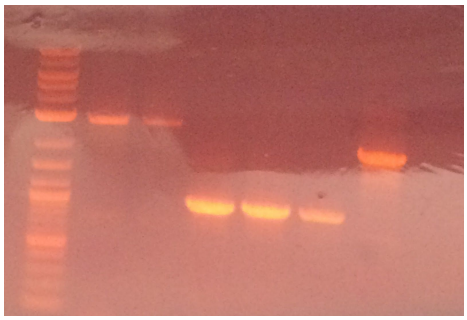
Objective: PCR

1. Set up PCR reactions for Sox R/S and 670.
    a. Made Master Mix

Material	Volume	Concentration
dH20	37.2 or 36.1 QS 50µL	-
5x ThermoPol Buffer	5 µL	-
dNTPs (50x)	1 µL	10 mM
iGEM 60 (20x)	2.5 µL	10 µM
iGEM 61 (20x)	2.5 µL	10 µM
pBAD/His/713 template	1.3 or 2.4 uL	
Or pBAD/HisB-iRFP 670 #2		
VentR DNA Polymerase	0.5 µL	100x stock

Thermocycling:

Temp. (°C)	Time	Cycle
95	5:00	
95	0:30	
57.9/58.9	0:30	35X
72	2:55/0:59	
72	10:00	
4	∞	



Objective: Hypoxia and iRFP 713 PCR.

Material	Volume	Concentration
dH20	QS 50µL	-
5x ThermoPol Buffer	5 µL	-
dNTPs (50x)	1 µL	10 mM
iGEM 45 (20x)	2.5 µL	10 µM
iGEM 46 (20x)	2.5 µL	10 µM
Hypoxia 670 / iRFP 713 Biobrick compatible #9 or pBAD/HisB-iRFP 670 #2		
VentR DNA Polymerase	0.5 µL	100x stock

Monday, July 18, 2016

Objective: PCR Sox R/S and pBAD/HiSB-iRFP 670.

1. PCR of Sox R/S #1. Vent, Primers iGEM 56 and 59. Elongation Time 2:55, Annealing temp 57.9.
2. Objective: PCR of pBAD/HisB-iRFP 670 #2. Vent, Primers 57, 58. Elongation time 59 s, Annealing temp 58.9.
3. Incubated 45 uL with DpnI at 37 C for 1 hour. Ran gel of 5 uL on 0.8% agarose gel for both PCRs.
4. Zymoclean and concentrated. Stored in Parts in Progress Box.

Objective: Golden Gate assembly Hypoxia iRFP 713 Biobrick Compatible.

1. Set up Golden Gate DNA Assembly.
    a. 0.61 uL 100 ng Hypoxia Backbone. 0.26 uL 44.6 ng iRFP 713. QS 15 uL dH2O

2. Zymocleaned and concentrated.
3. Transformed in XL1 EC by electroporation.
4. Incubated 1 h at 37 C with 1 mL SOC.
5. Plated 100 uL and the rest on LB/Cm plates.
6. Incubated 37 C for 16-18 hours.

Objective: Golden Gate assembly SoxR+S/iRFP670.

1. Set up Golden Gate DNA Assembly.
    a. 0.61 uL 100 ng SoxR/S Backbone. 1.9 uL 33.7 ng iRFP 713. QS 15 uL dH2O
2. Zymocleaned and concentrated.
3. Transformed in XL1 EC by electroporation.
4. Incubated 1 h at 37 C with 1 mL SOC.
5. Plated 100 uL and the rest on LB/Cm plates.
6. Incubated 37 C for 16-18 hours.

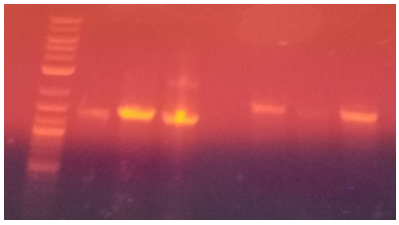
Objective: set up PCR for BioBrick Compatible iRFP 713.

1. Vent PCR protocol. Template: iRFP 713 Biobrick Compatible #9 or subsequent dilution (1:100 in E8). Primers: iGEM 60, 61. Annealing Temperature: 60.5 C, elongation Time: 1 min.

Tuesday, July 19, 2016

Objective: Set up Colony PCR and grow cultures for yesterday's plates

1. Vent PCR protocol with primers VF2 and VR for hypoxia iRFP 713, with primers iGEM 34 and iGEM 57 for SoxR+S/iRFP 670. Annealing temperature 51.6 C, length 1367 bp for hypoxia iRFP 713, annealing temperature 59.2 C, 1124 bp for SoxR+S/iRFP 670.
2. Ran 0.8% agarose gel at 110 V for 20 minutes for both.



Objective: Grow cultures of Biobrick compatible hypoxia iRFP670 for sequencing

1. Grow 4 3 mL cultures of colonies + LB/Cm.
2. Incubated for 16-18 hours at 37 C, 250 rpm.

Wednesday, July 20, 2016

1. Miniprep cultures from Tuesday with QIAprep Kit, send to Genewiz for Sequencing

Thursday, July 21, 2016

Sequencing for hypoxia713 #2 is good, sox670 #3 is good

Friday, July 22, 2016

Transformations:

1. SoxR/S 670 #3, hypoxia 670 #1, Sox R/S 713 #5, hypoxia 713 #2 in MG 1655 with Cm in Electroporation protocol on Benchling.
2. Ligation of BC 713-Constitutive #1 and #2 in XL1EC with Amp in Electroporation protocol on Benchling.

Sunday, July 24, 2016

1. Restreaked pETM6-H10 mCherry, Sox R/S 713, Sox R/S 670.
2. Plated Mg1655 on Cm (no growth expected)
3. Plated rest of ligation.
4. Grew cultures of Sox R/S 713 and 670.

Monday, July 25, 2016

Objective: miniprep mCherry, VioABECD, iRFP713, iRFP670 and send mCherry for sequencing

1. Miniprepped 2 samples each of mCherry, VioABECD, iRFP 713, iRFP 670 using Qiagen miniprep Spin Kit
2. Nanodropped all 8 samples
    a.

Sample	Concentration
mCherry #1	88.4 ng/uL
mCherry #2	104 ng/uL

    b.
3. Sent mCherry samples to Genewiz for sequencing, with T7 reverse primer (supplied by Genewiz)
4. Store iRFP 713 and iRFP 670 for possible future use.
5. Store VioABECD for future sequencing.

Tuesday, July 26, 2016

1. Removed previous plates. All plates had bacteria, stored at 4 degrees after parafilming.
2. No purple in violacein culture.

Objective: set up nitric oxide assay:

1. Added 200 uL Sox R/S iRFP 670 and 713 to corresponding 125 mL flasks with 50 mL LB/Cm (1:250 dilution).
2. Incubated at 37 degrees celsius and 250 rpm. Start time 10:40 am
3. Dilutions for NO assay: 10 mL culture + 32 uL 25 mM bilierdin, with 0 uM, 1 uM, 10 uM, 100 uM DETA/NO final concentration (Diethylenetriamine/nitric oxide adduct).

Objective: violacein arabinose test

1. Added 3 uL 1M arabinose to pETM6-E12-VioABECD in E. Rosetta
2. Incubated at 37 degrees celsius and 250 rpm. Start time 10:55 am (Expected purple color)
3. 12:35 PM added 3 uL 1M LPTG. Incubated as previous.
4. Streaked plates for VioABDE (BBa_K274003) and VioABCE (BBa_K274004)
    a. Stabbed agar stocks
    b. Streaked plates
    c. Let grow.

Objective: Miniprep of pETM6-H10-mCherry

1. Followed protocol from QIAprep Spin Miniprep Kit

Thursday, July 28, 2016

1. Nanodropped Constitutive 713 samples
2. Concentrations:

Constitutive 713 #1.1	202.1 ng/uL
Constitutive 713 #1.2	276.0 ng/uL
Constitutive 713 #1.3	292.4 ng/uL
Constitutive 713 #1.4	280.2 ng/uL
Constitutive 713 #2.1	238.4 ng/uL
Constitutive 713 #2.2	316.8 ng/uL
Constitutive 713 #2.3	316.7 ng/uL
Constitutive 713 #2.4	202.7 ng/uL

3. Sent to Genewiz for sequencing. Will check results tomorrow.

Friday, July 28, 2016

Resuspended primers: iGEM 67, 68, iGEM Gibson 1-10.

Sequencing results for G6 and H10 mCherry: pETM6-G6-mCherry #1 is good, #2 ok but not as good as 1. H10 #1 ok, #2 best.